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# Gene expression analysis in interstitial lung edema induced by saline infusion

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**Abstract**

To investigate the molecular events taking place during the development of hydraulic interstitial edema, we analyzed by microarray and conventional molecular techniques the variation of gene expression in lung from rabbits treated with slow-rate saline infusions. This analysis indicates that even a condition characterized by a small increase in extravascular water can have a significant influence on the inflammatory milieu. In this regard, cytokines, in particular TNFα, can be considered early mediators capable of inducing secondary effects on the injured tissue. Moreover, two MT1 genes were strongly up-regulated, data consistent with their role as protective molecules.


*Keywords:* Interstitial edema; Microarray; Cytokine; Lung

## 1. Introduction

Pulmonary edema is a condition characterized by an increased lung water content, which may be restricted to the interstitium or invade also the alveolar space. This latter condition is life-threatening, whereas interstitial edema is often associated with a mild clinical course and prompt resolution.

There are currently no data on the molecular mechanisms involved in the early phase of development of interstitial edema, although some results have been obtained in models involving more severe lung damages. In general, these analyses revealed variations in gene expression consistent with the occurrence of inflammation [1–7], oxidative stress [1,2], extracellular matrix (ECM) and cytoskeleton remodeling [1,8–10], cell proliferation [1,8,11], and apoptosis [12,13].

An experimental model of interstitial edema of a hydraulic type, induced in rabbit by slow-rate saline infusion [14], has been used in recent studies and proved useful to follow the biochemical and morphological events accompanying fluid accumulation in the extravascular space. Using this approach, it was demonstrated that early events of hydraulic edema are accompanied by matrix proteoglycan fragmentation due to activation of metalloproteases [15–19] and by a marked change in plasma membrane composition characterized by an increase in the lipid components typical of caveolae [20].

In the same animal model, treated by intravenous saline infusion with two different flow rates, we have now investigated the variation in lung gene expression. The obtained results revealed that in the early events of edema, mRNAs for inflammatory and protective molecules are already up-regulated, whereas a slight reduction of mRNAs for structural proteins was observed in the absence of clear signs of apoptosis.

## 2. Materials and methods

### 2.1. Lung tissue sample preparation

Lung tissue samples were obtained from 14 adult New Zealand rabbits [2.5 ± 0.6 (S.D.) kg body wt] anesthetized with a cocktail of 2.5 ml/kg of 50% (wt/vol) urethane and 40 mg/kg body wt of ketamine injected into an ear vein,

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Table 1
Summary of experimental conditions

| Group | Number of animals | Treatment (ml/kg·min) | Time of infusion (min) | Volume infused (ml/kg) | Anesthesia time (min) | W/D[§] | Hematocrit[§] |
|---|---|---|---|---|---|---|---|
| C | 3 | none | | 0 | 15 | 5.25 ± 0.34 | 40.7 ± 2.9 |
| S | 3 | none | | 0 | 180 | 5.3 ± 0.29 | 39.6 ± 2.7 |
| T1 | 4 | 0.5 | 180 | 90 | 180 | 6.35 ± 0.41* | 35.3 ± 1.2 |
| T2 | 4 | 1.5 | 180 | 270 | 180 | 6.78 ± 0.05* | 28.2 ± 2.3* |

C = control; S = sham; T = treated groups; W/D = wet to dry weight ratio; Hemotocrit = hematocrit value after saline infusion.
[§] Values ± S.D.
* $p < 0.05$ vs. control.

and tracheotomized to allow spontaneous breathing. Three animals were sacrificed shortly afterwards and used as controls (C); three more animals were kept under anesthesia for 180 min and used as shams (S). Eight animals received saline infusion at two different flow rates (T1 and T2), as reported in Table 1, to induce pulmonary interstitial edema. The hematocrit value was determined at the end of the experiments. An overdose of anesthesia was given, chest was opened as previously described [21] and the lungs were perfused with PBS plus 1000 U heparin till the perfusate was clear of blood. Lungs were excised from the chest, washed in sterile PBS, sliced, frozen in liquid nitrogen, and stored at −80 °C until use. To assess the wet to dry weight ratio (W/D), additional samples were taken from lungs, weighted fresh and after drying at 70 °C for at least 24 h.

### 2.2. DNA isolation and analysis

DNA was extracted as described by Sambrook et al. [22] and analyzed for fragmentation using 1% agarose gel electrophoresis and ethidium bromide staining.

Table 2
Rabbit probes derived from GenBank rabbit sequences or from alignment of GenBank sequences from different species (when the rabbit sequence was partial (*) or not available) and their PCR primers, conditions and product length

| Gene | Source | Genbank accession number | Primer | *T* (annealing) (°C) | Number of cycles | Probe length (bp) |
|---|---|---|---|---|---|---|
| IL1β | Rabbit | M26295 | F: 5′GGGCCTCAGGGGGAAGAATC 3′<br>R: 5′AGCAAGGGTCGGAGGAGTGG 3′ | 57 | 30 | 459 |
| IL6 | Rabbit | AF169176 | F: 5′AAGGCCAGCCCCGACAAAAC 3′<br>R: 5′GAGGCCGCGCAGGATGAAG 3′ | 59 | 35 | 483 |
| TNFα | Rabbit | M12845 | F: 5′CACCCTCAGATCAGCTTCTC 3′<br>R: 5′ATGATCCCAAAGTAGACCTG 3′ | 51 | 35 | 472 |
| IL10 | Rabbit | AF068058 | F: 5′GCAGGCAGAGTTCCACCAT 3′<br>R: 5′CCTTCCTCTTGCAGCTTGC 3′ | 54 | 35 | 475 |
| IFNγ | Rabbit | AB010386 | F: 5′GTTCTTACGGCTGTTACTGC 3′<br>R: 5′AGCGTCTGACTCCTTTTTCG 3′ | 54 | 35 | 423 |
| MT1 | Rabbit | M20015 | F: 5′ATGGACCCCAACTGCTCCTGC 3′<br>R: 5′TCAGGCGCAGCAGCTGCACTT 3′ | 62 | 35 | 186 |
| bFGF | Rabbit*<br>Human<br>Mouse<br>Rattus | L12034<br>NM002006<br>M30644<br>M22427 | F: 5′TGGCAGCCGGGAGCATCAC 3′<br>R: 5′AGCAGACATTGGAAGAAAAAGTAT 3′ | 57 | 35 | 458 |
| TGFβ1 | Rabbit*<br>Mouse<br>Rattus | AB020217<br>NM011577<br>NM021578 | F: 5′CAGGGCTTTCGCTTCAGTGCTCA 3′<br>R: 5′CAGACGCGCCCGGGTTGTGCT 3′ | 65 | 35 | 412 |
| MMP2 | Rabbit | D63579 | F: 5′GATGGCTTCCTCTGGTGCTC 3′<br>R: 5′CGGAAGTTCTTCGTGTAGGTG 3′ | 59 | 30 | 536 |
| Fibronectin | Rabbit | AF135404 | F: 5′GGCGCAGGACCAACAAAAACTAA 3′<br>R: 5′CTTCTCGGAGGGCTGACATTCT 3′ | 64 | 30 | 446 |
| Collagen I | Rabbit | D49399 | F: 5′GCAAGCGGCGGTGGTTACGAC 3′<br>R: 5′CAGCAGACGCATGAAGGCAAGTT 3′ | 63 | 30 | 474 |
| Dynein | Rabbit | AF008304 | F: 5′CCACGGTAACCATGTGCGAC 3′<br>R: 5′AGCAAGGCTGAAGACTCAGTC 3′ | 58 | 35 | 375 |
| α tubulin | Rabbit | AF200353 | F: 5′CCTTCTTCAGTGAGACAGGAGC 3′<br>R: 5′GGTGGTGTGGGTGGTGAGGAT 3′ | 62 | 35 | 431 |

### 2.3. RNA isolation

Total RNA was extracted from homogenized lungs using the Trizol reagent (Invitrogen, Milan, Italy) and further purified using the RNeasy Mini Kit (Qiagen, Milan, Italy).

### 2.4. cDNA synthesis

Total RNA was used to perform the synthesis of single- or double-stranded cDNA with the Superscript Choice system (Invitrogen) as described by the modified Affimetrix's protocol.

### 2.5. Labeling of microarray probes

To obtain a biotin-labeled antisense cRNA probe, 3.3 μg of double-stranded cDNA were in vitro transcribed with the RNA transcript Labeling Kit (Enzo Diagnostic Inc., Farmingdale, NY). Hybridization on the Affimetrix arrays U95A (Affimetrix Inc., Santa Clara, CA) and hybridization signal detection followed by data analysis using the Affimetrix microarray Suite 4.0 were performed at the facility of University of Milano-Bicocca (BIOPOLO). Each probe was hybridized twice on microarrays. Only genes showing a fold change of $\geq 2$ or $\leq -2$ in both hybridizations were considered.

### 2.6. Probes

Gene database searches and alignments were made at the National Center for Biotechnology Information (NCBI) using the GenBank network service. Probes were generated by PCR from control or edematous lungs cDNAs; PCR primers (Primm srl, Milan, Italy) and conditions are specified in Table 2. cDNAs fragments were cloned into pGEM-T Easy Vector System (Promega, Milan, Italy). Sequencing was performed using the Big Dye sequencing kit (Applera, Monza, Italy) and analyzed on a 3100 Genetic Analyzer (Applera). The obtained chromatograms were evaluated using the Sequence Analysis Program (Applera).

### 2.7. Northern blot analysis

Thirty micrograms of total RNA were size-fractionated on a 1% formaldehyde agarose gel, capillary transferred to a Nytran SuPerCharge membrane (Schleicher & Schuell,

Table 3
Genes differentially expressed in the T2 group compared to C group

| Category | Diff call | Fold change | Descriptions |
|---|---|---|---|
| Signal transduction | D | −3.3 | U94905: diacylglycerol kinase zeta mRNA, alternatively spliced |
| | D | −3.1 | HSU51004: protein kinase C inhibitor (PKCI-1) mRNA |
| | I | 2.9 | M14083: beta-migrating plasminogen activator inhibitor I mRNA |
| Growth factors | I | 2.2 | J04513: Human basic fibroblast growth factor (bFGF) 22.5, 21 and 18 kd protein mRNA |
| Transcription factors | D | −11.3 | M85234: nuclease sensitive element binding protein-1 mRNA |
| | I | 2.2 | V01512: Human cellular oncogene c-fos (complete sequence) |
| | I | 2.1 | X51345: jun-B mRNA |
| | I | 2.1 | AF043978: *Homo sapiens* paired-box transcription factor (PAX4) mRNA |
| Stress and DNA repair | D | −6.5 | Z23090: *H. sapiens* mRNA for 28-kDa heat shock protein |
| | D | −6.1 | Heat shock protein, 70 kDa |
| | D | −2.8 | HSU61397: ubiquitin-homology domain protein PIC1 mRNA |
| | I | 8.4 | K01383: metallothionein-I-A gene |
| | I | 4.5 | H68340: metallothionein-1f cDNA |
| | I | 2.1 | U38979: PMS2 related (hPMSR3) gene |
| Inflammation | I | 3.6 | AF035819: macrophage receptor MARCO mRNA |
| | I | 3.5 | M15330: Human interleukin 1-beta (IL1B) mRNA |
| | I | 2.8 | U86358: chemokine (TECK) mRNA |
| | I | 2.6 | AC004382: chemokine (STCP-1) |
| Cytoskeleton and matrix | D | −4.3 | U26162: myosin regulatory light chain mRNA |
| | D | −3.1 | AI540958: similar to cytoplasmic dynein, light polypeptide 1 |
| | D | −2.6 | X01703: gene for alpha-tubulin (b alpha 1) |
| | D | −2.5 | AB007937: mRNA for KIAA0468 protein, similar to N-syndecan |
| | D | −2.2 | AB014527: mRNA for KIAA0627 protein, similar to CLIP-associating protein 2 (CLASP) |
| Structure | D | −4.1 | U37230: Human ribosomal protein L23a mRNA protein synthesis |
| | D | −3.3 | AA877215: similar to RNP |
| | D | −2.2 | M37583: histone (H2A.Z) mRNA |
| Unknown | D | −4.5 | D80005: mRNA for KIAA0183 gene |
| | I | 2.1 | N35082 |
| | I | 2 | AB011109: mRNA for KIAA0537 protein |

D = decreased expression; I = increased expression.

Keene, NH). Membranes were hybridized overnight at 60 °C using cDNA probes labeled with [$\alpha^{32}$P]dCTP (800 mCi/mmol, Amersham, Milan, Italy) by means of random priming (Megaprime kit, Amersham). Membranes were exposed to Hyperfilm MP (Amersham) at − 80 °C using intensifying screens and the autoradiographs were quantified by means of computer-assisted densitometry.

### 2.8. Semiquantitative RT-PCR

Twelve microliters of each cDNA were used to seed a 80-μl PCR mixture using the primers (16 pmol) and temperature of annealing described in Table 2. Twenty-two microliters of the reaction mixture were withdrawn after 20, 25, and 30 cycles, separated on a 2% agarose gel and transferred to a Nytran SuPerCharge membrane (Schleicher & Schuell). Hybridizations were performed at 42 °C overnight with 20 pmol of specific internal oligonucleotides (Primm) labeled by the T4 polynucleotide kinase (Amersham) with 5 μl of [$\gamma^{32}$P]dCTP (800 mCi/mmol, Amersham). Membranes were exposed to Hyperfilm MP (Amersham) and evaluation of the hybridization signal was performed at 20, 25, and 30 cycles. The autoradiographs were quantified by means of computer-assisted densitometry to monitor the progress of product accumulation.

### 2.9. Statistical analysis

Statistical analysis was performed using the mean values ± S.D. of the densitometric data obtained in each treated group (four animals) vs. the control group (six animals), by Northern blot and RT-PCR. In the latter case, the values considered were those obtained in cycles in which exponential amplification was detected. The statistical significance of the variation in mRNA expression, related to different treatments, was evaluated by the analysis of variance (ANOVA test), and by multiple comparison procedure (Tukey test). Before the statistical analysis, each densitometric value was normalized to G3PDH.

## 3. Results

### 3.1. Preliminary sample analysis

Table 1 reports the W/D and the hematocrit values measured in the animals classified according to the different groups (C as controls, S as shams, T1 and T2 as treated groups). No significant variations were observed between the control and the sham animals regarding W/D and hematocrit values. Conversely, both treatments caused a significant increase in the W/D compared to shams and controls. The decrease in the hematocrit values was significant only in the T2 animals. Tissues from control and treated animals did not exhibit apoptosis detectable by DNA laddering (not shown).

### 3.2. Microarray analysis

Most of the information concerning the macromolecular perturbations and the alterations in microvascular pulmonary interstitial fluid balance were gathered in the rabbit, which represents an adequate experimental model to mimic the early events that occur in the pathophysiology of human lung edema. However, this rabbit model shows a limitation represented by the partial knowledge currently available on its genome. Due to this limitation, in order to obtain some insights into the modulation of gene transcription in our experimental model, T2 and sham transcripts were compared using the U95A human genome high-density oligonucleotide GeneChip array. Among the 12,600 tested genes, considering a minimum fold change of $\geq 2$ or $\leq -2$, only 29 showed a differential expression (15 decreased and 14 increased) (Table 3). The maximal decrease (11.3-fold lower than control) was observed for a nuclease-sensitive element binding protein 1 (a putative transcription factor), and the

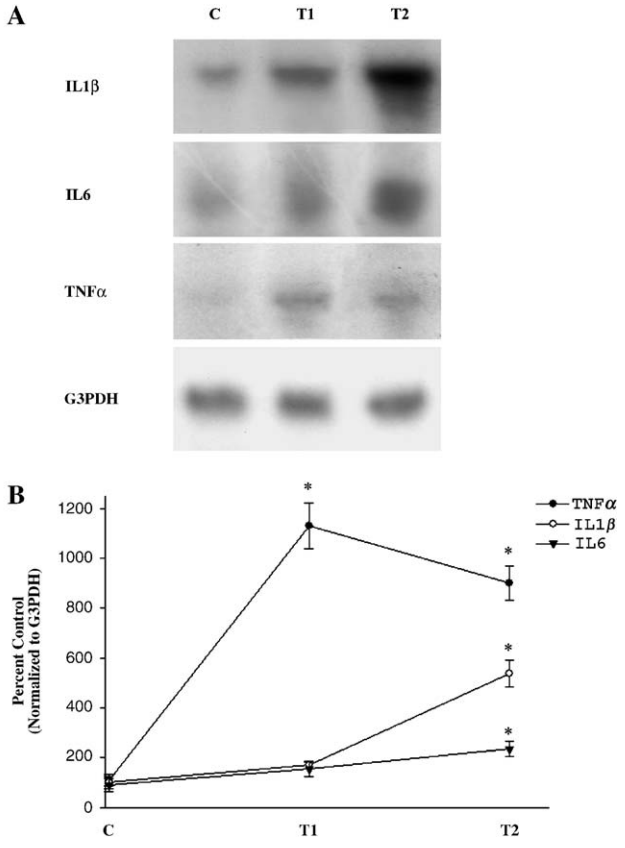


Fig. 1. (A) Northern blot analysis of RNA from control (C) and treated animals (T1, 0.5 ml/kg·min for 180 min; T2, 1.5 ml/kg·min for 180 min). RNA samples were fractionated on 1% denaturing agarose gels, transferred to nylon membranes and hybridized to $^{32}$P-labeled cDNA probes specific for IL1β, IL6, TNFα, and G3PDH. (B) Densitometric analysis of Northern blot hybridizations for IL1β, IL6, and TNFα mRNA expression (values are means ± SD, $n=6$ or 4 for controls and treated rabbits, respectively). mRNA levels are expressed as percent of control values, normalized to G3PDH. *$P<0.05$ vs. controls.

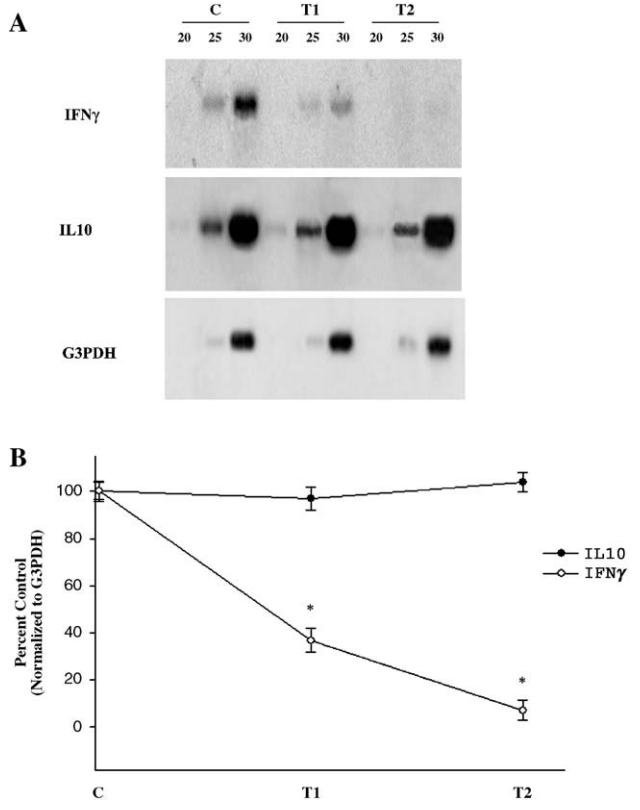


Fig. 2. (A) Semiquantitative RT-PCR of IFNγ, IL10, and G3PDH. cDNAs were retrotranscribed from total RNA of control (C) and treated rabbits (T1 and T2) and used as templates in the PCR reaction. Aliquots of the amplification reactions were taken at 20, 25, and 30 cycles, fractionated on 2% agarose gels and transferred to nylon membranes. PCR samples were hybridized to $^{32}$P-labeled synthetic oligonucleotides specific for IFNγ, IL10, and G3PDH, respectively. (B) Densitometric analysis of semiquantitative RT-PCR experiments for IFNγ and IL10 (values are means ± SD, $n=6$ or 4 for controls and treated rabbits, respectively). PCR product amounts, normalized to G3PDH, are expressed as percent of control values. *$P<0.001$ vs. controls.

largest increase (8.4-fold) for metallothionein 1A. The identified genes were grouped according to the function or their belonging to a common pathway. The analysis evidenced seven categories, namely: signal transduction (3 genes, 2 decreased and 1 increased), growth factors (1 gene, increased), transcription factors (4 genes, 1 decreased and 3 increased), stress and DNA repair (6 genes, 3 decreased and 3 increased), inflammation (4 genes, increased), cytoskeleton and matrix (5 genes, decreased) and structural components (3 genes, decreased). Three genes corresponded to sequences with unknown function (2 increased and 1 decreased), one of which (AB011109) showed a moderate similarity with a protein kinase.

### 3.3. Analysis by conventional techniques of gene expression in control and treated animals

To validate some of the major evidence of the microarray data, and to extend the gene expression analysis to other possible genes not evidenced by the gene chip technique, all probes listed in Table 2 were evaluated by Northern blot or semiquantitative RT-PCR.

#### 3.3.1. Control and sham animals

To verify the absence of an anesthesia effect on RNA synthesis, the probes were first used to compare gene expression in control and sham animals. The Northern blots or semiquantitative RT-PCR analysis (not shown) did not evidence any significant variation between the two groups, and therefore all the animals were considered as a homogeneous control group (indicated as C).

#### 3.3.2. Cytokines

mRNA expression of the proinflammatory cytokines IL1β, IL6, and TNFα was quantified by Northern blot analysis in all control and treated lungs (T1 and T2). The

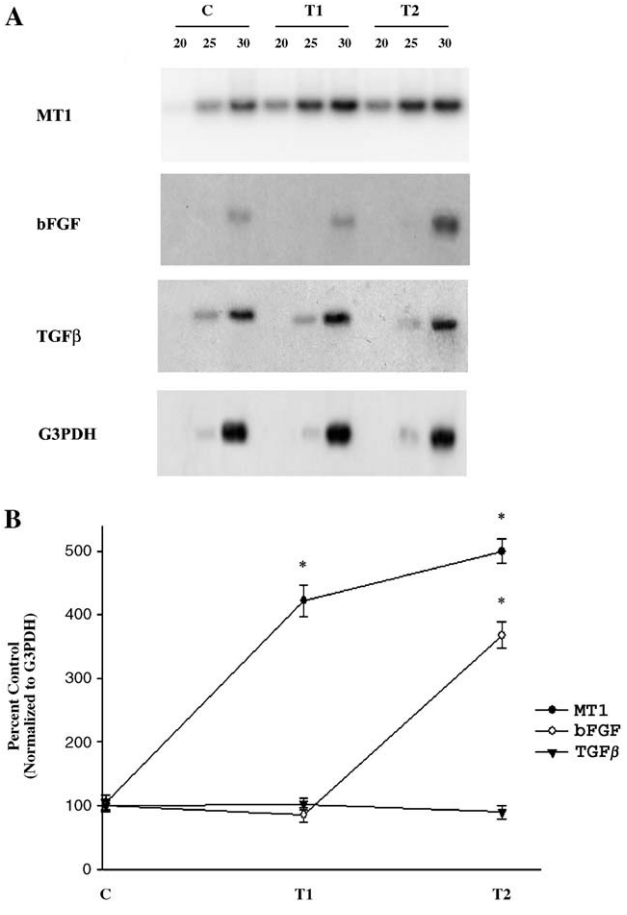


Fig. 3. (A) Semiquantitative RT-PCR of MT1, bFGF, TGFβ, and G3PDH. cDNAs were retrotranscribed from total RNA of control (C) and treated rabbits (T1 and T2) and used as templates in the PCR reaction. Aliquots of the amplification reactions were taken at 20, 25, and 30 cycles, fractionated on 2% agarose gels and transferred to nylon membranes. PCR samples were hybridized to $^{32}$P-labeled synthetic oligonucleotides probes. (B) Densitometric analysis of semiquantitative RT-PCR for MT1, bFGF, and TGFβ (values are means ± SD, $n=6$ or 4 for controls and treated rabbits, respectively). PCR product amounts, normalized to G3PDH, are expressed as percent of control values. *$P<0.001$ vs. controls.

hybridization pattern obtained in one animal of each group is shown in Fig. 1A; all the animals belonging to the same group gave very similar hybridization results. As demonstrated by the densitometric analysis (Fig. 1B), IL1β and IL6 mRNAs increased (approximately 5- and 2.5-fold, respectively) only in the interstitial edema induced by saline infusion of 1.5 ml/kg·min (T2) as compared to the control group (C) ($P<0.001$ and $P<0.05$). For IL1β, the data were consistent with those obtained by the microarray analysis. TNFα mRNA expression increased significantly (11- and 9-fold, respectively) in both treatments ($P<0.001$ for both).

The evaluation of IFNγ and IL10 mRNA expression by semiquantitative RT-PCR is reported in Fig. 2A; data representative of those obtained analyzing all the animals in each group. Densitometric analysis (Fig. 2B) evidenced a marked decrease in IFNγ mRNA expression both in T1 and T2 samples ($P<0.001$ for both groups), whereas the mRNA expression of the anti-inflammatory cytokine IL10 did not change significantly.

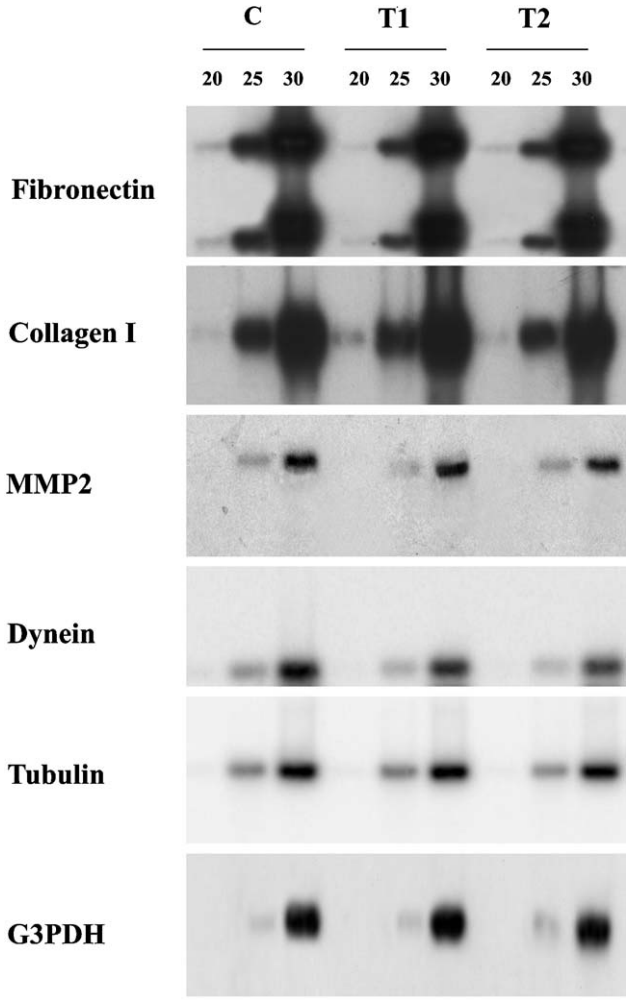


Fig. 4. Semiquantitative RT-PCR of fibronectin, collagen I, MMP2, dynein, tubulin, and G3PDH cDNAs were retrotranscribed from total RNA of control (C) and treated rabbits (T1 and T2) and used as templates in the PCR reaction. Aliquots of the amplification reactions were taken at 20, 25, and 30 cycles, fractionated on 2% agarose gels and transferred to nylon membranes. PCR samples were hybridized to $^{32}$P-labeled synthetic oligonucleotides probes. The data shown are representative of the results obtained in all the treated and control animals.

### 3.3.3. Growth factors and stress response molecules

We analyzed the gene expression of two growth factors, TGFβ and bFGF, and of the stress response molecule MT1 by semiquantitative RT-PCR (Fig. 3A). The densitometric analysis (Fig. 3B) did not evidence any substantial variation in TGFβ mRNA levels; conversely, bFGF mRNA expression significantly increased only in T2 samples ($P<0.001$), thus confirming the data obtained by the microarray assay. MT1 mRNA expression showed a marked increase in both treatments ($P<0.001$), thus again confirming the data observed by the microarray analysis performed using the T2 sample.

### 3.3.4. Cytoskeleton and matrix molecules

Gene expression of mRNAs for fibronectin, α2 type I collagen, MMP2, dynein, and α tubulin was evaluated by semiquantitative RT-PCR analysis (Fig. 4). The densitometric analysis (not shown) evidenced only a slight reduction for dynein mRNA in T2 (about 25%), but it did not reach statistical significance. In this case, the RT-PCR data did not parallel those obtained by the microarray analysis, where the fold change in tubulin and dynein expression was much higher.

## 4. Discussion

Pulmonary interstitial edema can be defined as the condition where only an increase of extravascular fluid is observed, without the presence of water in the alveolar spaces [21]. This situation represents a transition between the physiological condition and severe lung edema, thus being a good system to derive important information on the pathophysiology of both edema development and recovery.

In this paper, by manipulating infusion rates, we caused an increase in extravascular water reflected by an augmented W/D in the order of 20–30% and no alveolar fluid was present as confirmed by microscopy. In these experimental conditions we investigated, first by an oligonucleotide human microarray system, the variation in gene expression using the mRNA population from a lung rabbit with the highest value of W/D (T2) as a probe. Twenty-nine genes showed an appreciable modulation in their level of expression. Interestingly, this analysis revealed a general up-regulation of genes involved in inflammation (IL1β and chemokines), whereas mRNAs for the cytoskeleton, ECM, and structural components were all slightly down-regulated. The genes belonging to the other categories showed a variable transcriptional behavior. The microarray hybridization was carried out using a rabbit lung mRNA population on a set of human genes, and thus a possible underevaluation of the overall gene expression could be hypothesized,

mainly due to the divergence between the 3′ end of human and rabbit mRNAs. Furthermore, the microarray analysis was performed on one T2 and control animal. Thus, to verify the microarray results, and to rule out possible experimental variations, we further analyzed, with conventional molecular techniques using the mRNA populations from all controls and T2 lungs, some of the genes that were modulated (IL1β, MT-1, bFGF, dynein, and tubulin) and some that were not (TNFα, IL6, IFNγ, MMP2, collagen, fibronectin, TGFβ, and IL10). In this regard, eight out of the thirteen analyzed genes confirmed the microarray data. Conversely, the two proinflammatory cytokines, IL6 and TNFα, showed a marked up-regulation, INFγ was down-regulated, and dynein and tubulin (detected as down-regulated by microarray) did not show appreciable changes.

The comparison between T2 and T1 animals showed that the treated animals exhibiting the lower W/D ratio (T1) were characterized by variations in expression of fewer genes. In particular, in the T1 group, only TNFα and MT1 were markedly up-regulated while IFNγ was down-regulated. Thus, the early phases of hydraulic interstitial edema development are characterized by a proinflammatory response, which is concomitant with the appearance of signals of stress and repair, probably correlated to intra- and extracellular damages and/or remodeling. Interestingly, the observed up-regulation of the plasminogen activator inhibitor 1, a molecule that was proposed to promote ECM deposition in the airways of a murine asthma model [23], seems consistent with the previously demonstrated ECM remodeling in interstitial edema [16–18]. The induction of the proinflammatory response agreed with a slight up-regulation of transcription factors such as junB and cfos, which are the predominant components of the AP1 complex. A recent report suggests that in acute lung injury due to immune complexes, cytokine gene expression requires the participation of the AP1 complex [24]. Thus, a possible cascade of events includes the activation of TNFα, IL1β, and other cytokines and chemokines. Endothelial cells could be the putative producers of mediators of the proinflammatory response since no alveolar fluid was present in our model and, therefore, activation of pulmonary macrophages, residing on the alveolar surface, should not have occurred. The inflammatory response could then propagate from endothelial to other lung cell types. Furthermore, IL1β and TNFα are known to be responsible for the increased production of secondary cytokines, such as IL6 [25]. Our results thus suggest that similar events occur in the early phase of development of interstitial edema as supported by the detection of increased TNFα mRNA expression, even in the T1 animals.

In both treatments, the down-regulation of IFNγ was observed. It has been shown that IFNγ is involved in increased permeability of endothelial barrier [26] and induction of apoptosis [27]. Thus, the down-regulation displayed in the present model could suggest a possible mechanism that helps to maintain the endothelial barrier structure intact, and may counteract the opposite effect caused by the increased in TNFα and IL1β. The up-regulation in the T2 animals of bFGF, a molecule involved in the angiogenesis [28], supports the hypothesis of a lung vasculature remodeling during volume overload.

Similarly to findings in more severe lung injuries [1], two MT1 genes markedly increased their expression, these data being consistent with their role as antioxidant agents. Conversely, genes for factors involved in the preservation of protein damage, i.e. two heat shock proteins and an ubiquitin-like protein, were down-regulated. Although unexpected, this reduction in their mRNA level could be related to the fact that heat shock proteins induction inhibits the expression of proinflammatory cytokines [29].

The transcription of other genes, such as those for MMPs, was not affected. Since a previous study demonstrated an early activation of latent proforms of these proteins [19], it is possible that similar posttranslational mechanisms are also used for other genes, allowing a faster response to the changes in the cellular microenvironment.

In summary, the modulation of gene expression in lung edema obtained using heterologous system (i.e. rabbit vs. human) was partially confirmed by the subsequent evaluation of gene expression using rabbit probes. The suggestion from this study stems from endothelial cell activation; stimuli affecting mRNA modulation may originate either on luminal side, due to shear stress and vascular distension, or at tissue level, due to parenchymal forces caused by matrix imbibition [18] obtained by a ‘‘pure’’ hydraulic edema model. Accordingly, we suggest that endothelial mechanotransduction represents the first event leading to the production of cytokines, which could be considered the early mediators able to induce secondary effects including interstitial matrix turnover.

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